

## Editorial

## A starting point for structure function relationships in the canine pulmonary veins

Brenda R. Kwak\*, Dipen C. Shah, François Mach

*Foundation for Medical Research, Division of Cardiology, University Hospital, 64 Avenue de la Roseraie, 1211 Geneva 4, Switzerland*

Received 27 June 2002; accepted 27 June 2002

*See article by Verheule et al. [6] (pages 727–738) in this issue.*

The recent demonstration of the effectiveness of catheter ablation of the pulmonary veins as a curative treatment for paroxysmal atrial fibrillation (AF) has prompted efforts to examine in detail the sleeves of atrial myocardium encasing their left atrial ends [1]. Multi-electrode catheter mapping of spontaneous episodes of AF in patients show that the majority (>90%) of paroxysms begin with earliest activation at the ostium or within the pulmonary veins [2]. Consistent initiation of paroxysms of AF from tissue in this region reinforces the probability of distinctive structural or functional characteristics being responsible for such anatomical localization.

Activation mapping within the confines of the pulmonary veins is limited by their diameter (about 15–17 mm), complex branching structure as well as the limitations of access through a transseptal puncture. A preformed loop shaped multi-electrode catheter positioned orthogonal to the venous long axis, at the relevant ostium or within the vein, provides a slice of circumferential activation [3], while the integration of an additional longitudinal mapping ability e.g. with a basket catheter provides the maximal currently and clinically possible mapping coverage of the vein. Nevertheless, this leaves unmapped territory in the first order branches. Though decremental conduction, spontaneous activity as well as exit block have been documented within the veins or at their junction with the left atrium using the above catheters [2,4], there is limited detail about the electrophysiology of this region. A recent report described basket catheter mapping of a tachycardia originating in the superior vena cava—a thoracic vein with a structure similar to the pulmonary veins [5]. Activation

compatible with a physically small circus movement reentry circuit (based on nonuniform anisotropy) was found to be confined to the myocardial sleeve of the superior vena cava with intermittent conduction to the right atrium. During both tachycardia as well as programmed stimulation in sinus rhythm, evidence of marked slow conduction was found; notably, conduction was faster in the long axis of the vein as compared to perpendicularly. In contrast to such abnormal impulse conduction, the first beat(s) of the initiation of a paroxysm of AF displays characteristics compatible with triggered activity though abnormal automaticity cannot be ruled out. These abnormal impulses likely trigger a reentrant arrhythmia, that spreads to involve the atria. Thus, a combination of arrhythmia mechanisms is very likely responsible for AF. The myocardial fiber arrangement, their coupling by means of gap junctions and the intervening fibrous tissue certainly affect the milieu for reentry by affecting the wavelength of the tissue. Whether they similarly affect or promote abnormal impulse generation is not clear though some degree of protection or isolation from normal currents of surrounding cells is felt to be necessary for abnormal automaticity.

In this context we can view the results of Verheule et al. [6] as a starting point for understanding the role of the pulmonary veins in initiating and maintaining atrial fibrillation. At both light and electron microscopic levels, they did not find any significant differences in myocyte morphology between pulmonary vein and atrial myocardium. Significantly, the authors found no structural evidence of abnormal nodal like ‘pale cells’ which have been proposed as the origin of abnormal impulse generation [7]. The authors believe that based on the results of connexin immunolabelling, the venous myocardium is tightly coupled to atrial myocardium and remark only upon the spiral arrangement of myofibers as well as the lesser degree of staining for one type of gap junction protein (see below). Their histological findings are essentially in tune with what

---

\*Corresponding author. Tel.: +41-22-382-7238; fax: +41-22-382-7245.

E-mail address: [brenda.kwakchanson@medecine.unige.ch](mailto:brenda.kwakchanson@medecine.unige.ch) (B.R. Kwak).

others have found: the thickness of the myocardial sleeve decreased towards the pulmonary end with bundles of myocardial fibers being separated by fibrous tissue only at this level. They also found sharp transitions in fiber arrangements which have in fact been found to correlate with zones of activation delay and even conduction block [8]. Closer to the left atrial end, Verheule et al. describe more longitudinally oriented fibers on the epicardial side whereas a circumferential pattern was observed close to the endocardium essentially throughout the length of the myocardial sleeve. Anisotropic conduction delays favouring reentry may therefore be prominent at the overlap region of these two fiber orientations.

Gap junctions are clusters of transmembrane channels that connect the cytoplasmic compartments of neighboring cells, thus enabling the direct exchange of ions and small molecules. Molecular cloning studies have demonstrated that gap junction channels are formed by members of a family of related proteins called connexins (Cx) in vertebrates. There are about 20 different connexin types in the human and mouse genome [9,10]. The commonly used nomenclature distinguishes Cx by their molecular mass deduced from their respective sequences. The 43-kDa protein Cx43 is the most abundant in the heart and has been found in almost all parts of the organ, with the exception of the sinoatrial and atrioventricular nodes. The second important cardiac connexin, Cx40, is specifically present in the atria and in the ventricular conduction system (for reviews, see Refs. [11,12]). Other cardiac connexins display a restricted expression pattern. Thus, Cx45 expression has been reported in nodal tissues and the conduction system [13], Cx46 in the sinoatrial node [14], Cx50 in atrioventricular valves [15], and finally Cx37 in the endocardial endothelium [10]. It is well known that each type of gap junctional channel has unique inherent gating properties, permeabilities to various molecules and ions as well as regulation by second messengers [16–19]. In addition, Cx are dynamic proteins with half-lives ranging from 1 to 5 hours, indicating that gap junction channels are fully exchanged several times per day [20,21]. This provides another mechanism to regulate direct cytoplasmic cross-talk between cells under normal or pathological conditions.

In their search for a substrate for focal activity leading to AF, Verheule et al. [6] investigated gap junctions around the veno-atrial transition using immunostainings for Cx43 and Cx40. In the past decade, numerous publications have reported changes in ventricular gap junction expression levels and patterns that may predispose to the common and often fatal arrhythmias observed with acute ischemia, chronic myocardial infarction, hypertrophy and heart failure [22–25]. Indeed, two main forms of gap junction abnormality are frequent in various human heart diseases: a redistribution of Cx43 from end-to-end-located intercalated disks to the lateral cell borders and a global reduction of Cx43 throughout the myocardium. Whether these gap

junction abnormalities may lead to the slow conduction and/or heterogeneous wavefront propagation characteristic for micro-reentrant electrical circuits underlying most of these cardiac arrhythmias remains, however, a matter of debate [26]. In their study of the myocardial sleeve, Verheule et al. [6] described large gap junctions at intercalated disks, abundant expression of Cx43 and reduced Cx40 expression compared to the left atrium. The authors therefore conclude that conduction in pulmonary veins is maintained by abundant Cx43, and that it is likely tissue geometry, i.e. the circumferential orientation of the myocytes in the myocardial sleeves, rather than gap junctional communication that may provide a substrate for reentry. Although this conclusion seems justified on the basis of their data, a recent study by Dupont et al. [27] describing that patients prone to develop AF after surgery appear to have higher levels of Cx40 in the atrial myocardium than those who do not, should be kept in mind.

The current study of Verheule et al. [6] describes a homogeneous expression of low levels of Cx40 and high levels of Cx43 in the pulmonary vein sleeves. This is in surprising contrast to an earlier report of Yeh and colleagues [28] who have studied for the same reason the myocardial sleeve in the canine superior vena cava. In addition to differences in assembly of cardiomyocytes and their spatial orientation, they observed areas of atypical connexin expression featuring a center rich in Cx43 labeling surrounded by a periphery of scattered tiny Cx40 labels. Consequently, these authors suggest that tissue geometry and variation in connexin distribution could potentially combine to create a substrate for heterogeneity of electrical coupling. Increase in heterogeneity of gap junction distribution has also been observed in a well-defined animal model of sustained AF, as originally described by Wijffels et al. (AF begets AF [29]). In this study, the development of heterogeneity of Cx40 distribution displayed a strong correlation with the time course of stabilization of AF in the instrumented goats, suggesting the involvement of gap junction remodeling in the pathogenesis of sustained AF [30]. Interestingly, radiofrequency catheter ablation has been shown to eliminate pacing-induced sustained AF in combination with a reduction in Cx43 in dogs atria [31]. Thus, as already suggested by Spach and Starmer in 1995, therapeutic strategies aimed at altering the topology of gap junctions may produce better results than conventional pharmacological anti-arrhythmic therapy [32]. In this respect it is worth mentioning the recent development of synthetic peptides acting on gap junctional coupling (such as AAP10 [33,34]) that appear to be effective in reducing dispersion of action potential duration in the ischemic isolated rabbit heart.

Since no animal model of spontaneously initiating AF exists, it is less likely that one would find significant abnormality in normal canine pulmonary veins. However, the role of thoracic veins in artificially induced but

spontaneously maintained AF is important given that there is experimental data showing that surgical isolation of the pulmonary veins (and atrial appendages) rendered AF induction impossible in a sheep theophylline infusion model [35]. Heart failure models producing structural remodeling may be more relevant clinically and more likely to show evidence of pathological changes. Altogether, this study of canine pulmonary veins provides a useful starting reference point of normalcy and will without doubt serve as a detailed basis for future studies towards the mechanisms for focal AF in various types of pathological models.

## Acknowledgements

This work was supported by grants from the Swiss National Science Foundation (MHV #3234-066311.01 to BK and #3200-065121.01 to FM).

## References

- [1] Haissaguerre M, Jais P, Shah DC. Electrophysiological end point for catheter ablation of atrial fibrillation initiated from multiple pulmonary venous foci. *Circulation* 2000;101:1409–1417.
- [2] Haissaguerre M, Jais P, Shah DC. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med* 1998;339:659–666.
- [3] Haissaguerre M, Shah DC, Jais P. Electrophysiological breakthroughs from the left atrium to the pulmonary veins. *Circulation* 2000;102:2463–2465.
- [4] Chen SA, Hsieh MH, Tai CT. Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: electrophysiological characteristics, pharmacological responses and effects of radiofrequency ablation. *Circulation* 1999;100:1879–1886.
- [5] Shah DC, Haissaguerre M, Jais P. High resolution mapping of tachycardia originating from the superior vena cava: evidence of electrical heterogeneity, slow conduction, and possible circus movement reentry. *J Cardiovasc Electrophysiol* 2002;13:288–392.
- [6] Verheule S, Wilson EE, Arora R, et al. Tissue structure and connexin expression of canine pulmonary veins. *Cardiovasc Res* 2002;55:727–738.
- [7] Masani F. Node-like cells in the myocardial layer of the pulmonary vein of rats: an ultrastructural study. *J Anat* 1986;145:133–142.
- [8] Hocini M, Ho SY, Kawara T. Electrical conduction in canine pulmonary veins. Electrophysiological and anatomic correlation. *Circulation* 2002;105:2442–2448.
- [9] White TW, Paul DL. Genetic diseases and gene knockouts reveal diverse connexin functions. *Annu Rev Physiol* 1999;61:283–310.
- [10] Willecke K, Eiberger J, Degen J. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem* 2002;383:725–737.
- [11] Gros DB, Jongsma HJ. Connexins in mammalian heart function. *Bioessays* 1996;18:719–730.
- [12] Severs NJ, Rothery S, Dupont E. Immunocytochemical analysis of connexin expression in the healthy and diseased cardiovascular system. *Microsc Res Tech* 2001;52:301–322.
- [13] Coppen SR, Severs NJ, Gourdie RG. Connexin45 (alpha 6) expression delineates an extended conduction system in the embryonic and mature rodent heart. *Dev Genet* 1999;24:82–90.
- [14] Verheule S, van Kempen MJ, Postma S. Gap junctions in the rabbit sinoatrial node. *Am J Physiol* 2001;280:H2103–2115.
- [15] Gourdie RG, Green CR, Severs NJ. Immunolabelling patterns of gap junction connexins in the developing and mature rat heart. *Anat Embryol* 1992;185:363–378.
- [16] Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. *Eur J Biochem* 1996;238:1–27.
- [17] Elfgang C, Eckert R, Lichtenberg-Frate H. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. *J Cell Biol* 1995;129:805–817.
- [18] Kwak BR, Hermans MM, De Jonge HR. Differential regulation of distinct types of gap junction channels by similar phosphorylating conditions. *Mol Biol Cell* 1995;6:1707–1719.
- [19] Cao F, Eckert R, Elfgang C. A quantitative analysis of connexin-specific permeability differences of gap junctions expressed in HeLa transfectants and *Xenopus* oocytes. *J Cell Sci* 1998;111:31–43.
- [20] Laird DW, Puranam KL, Revel JP. Turnover and phosphorylation dynamics of connexin43 gap junction protein in cultured cardiac myocytes. *Biochem J* 1991;273:67–72.
- [21] Beardslee MA, Laing JG, Beyer EC. Rapid turnover of connexin43 in the adult rat heart. *Circ Res* 1998;83:629–635.
- [22] Smith JH, Green CR, Peters NS. Altered patterns of gap junction distribution in ischemic heart disease. An immunohistochemical study of human myocardium using laser scanning confocal microscopy. *Am J Pathol* 1991;139:801–821.
- [23] Peters NS, Green CR, Poole-Wilson PA. Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts. *Circulation* 1993;88:864–875.
- [24] Sepp R, Severs NJ, Gourdie RG. Altered patterns of cardiac intercellular junction distribution in hypertrophic cardiomyopathy. *Heart* 1996;76:412–417.
- [25] Dupont E, Matsushita T, Kaba RA. Altered connexin expression in human congestive heart failure. *Mol Cell Cardiol* 2001;33:359–371.
- [26] Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. *Circ Res* 2000;86:1193–1197.
- [27] Dupont E, Ko Y, Rothery S. The gap-junctional protein connexin40 is elevated in patients susceptible to postoperative atrial fibrillation. *Circulation* 2001;103:842–849.
- [28] Yeh HI, Lai YJ, Lee SH. Heterogeneity of myocardial sleeve morphology and gap junctions in canine superior vena cava. *Circulation* 2001;104:3152–3157.
- [29] Wijffels MC, Kirchhof CJ. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation* 1995;92:1954–1968.
- [30] Van der Velden HM, Ausma J, Rook MB. Gap junctional remodeling in relation to stabilization of atrial fibrillation in the goat. *Cardiovasc Res* 2000;46:476–486.
- [31] Elvan A, Huang XD, Pressler ML. Radiofrequency catheter ablation of the atria eliminates pacing-induced sustained atrial fibrillation and reduces connexin 43 in dogs. *Circulation* 1997;96:1675–1685.
- [32] Spach MS, Starmer CF. Altering the topology of gap junctions: a major therapeutic target for atrial fibrillation. *Cardiovasc Res* 1995;30:337–344.
- [33] Dhein S, Manicone N, Muller A. A new synthetic antiarrhythmic peptide reduces dispersion of epicardial activation recovery interval and diminishes alterations of epicardial activation patterns induced by regional ischemia. A mapping study. *Naunyn Schmiedeberg Arch Pharmacol* 1994;350:174–184.
- [34] Muller A, Gottwald M, Tudyka T. Increase in gap junction conductance by an antiarrhythmic peptide. *Eur J Pharmacol* 1997;327:65–72.
- [35] Fieguth HG, Wahlers T, Borst HG. Inhibition of atrial fibrillation by pulmonary vein isolation and auricular resection—experimental study in a sheep model. *Eur J Cardiothorac Surg* 1997;11:714–721.